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COMPARATIVE EVALUATION AND QUANTIFICATION OF CIRCUM -GINGIVAL PORPHYROMONAS GINGIVALIS IN BETEL NUT CHEWERS AND NON CHEWERS - MOLECULAR MICROBIOLOGICAL STUDY

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ABSTRACT

Background: There is statistical evidence relating periodontal condition and betelnut chewing, However few survey in this region clearly shows the elevated percentage of people suffering from periodontitis belong to betelnut chewers compared to non chewers Also there is no much study on the periodontal pathogen Porphyromonas gingivalis as far its quantification between betel nut chewers and non chewers. The aim of this study is to quantify and compare circum -gingival porphyromonas gingivalis in betel nut chewers and non chewers. **Method:** Ten subjects were included in each group of betel and non betel chewers to determine the quantification of circum -gingival porphyromonas gingivalis by real time PCR. **Result:** The test shows that there is no significant difference in the number of cells per ml among the groups. The study result shows that there is no association between the groups and the bacterial cell count between betel and non betel chewers. **Conclusion:** It is found that there is no significant difference between betel nut chewers and nonchewers on quantification and comparison of circumgingival porphyromonas gingivalis.

INTRODUCTION

Periodontitis is one among the common diseases of the oral cavity. It is characterized by connective tissue destruction followed by an inflammatory host response secondary to periodontal bacterial infection.[1] Globally 5 – 20 % of adult population is affected by severe periodontal disease resulting in tooth loss.[2] Also, it is the 11th most prevailing disease globally than cardiovascular disease.[3] The incidence of periodontitis increases with age, and

therefore the prevalence rises steeply in adults aged 50 to 60 years.[4] Periodontitis is a multifactorial disease that happens by the infiltration of bacteria into the periodontium thereby progressing gingival inflammation.[5] One of the factor s that influence periodontal disease is the dental plaque. Most of the periodontitis are a result of plaque - induced gingivitis.[6] Oral biofilm has several roles in the development of various diseases of the oral cavity and throat, resulting in caries, periodontal diseases, endodontic infections, alveolitis, and tonsillitis. Dental plaque (also called microbial plaque, oral biofilm, and dental biofilm) has a highly organized different microbial community that is attached to the hard tooth structure surfaces.[7] Porphyromonas gingivalis is a gram negative, anaerobic, non-motile, asaccahrolytic and black pigmented rod, seen in subgingival plaque. It is one of the key pathogens in chronic periodontitis. Host tissue destruction happens when the levels of Porphyromonas gingivalis along with other bacteria reach a critical threshold, Porphyromonas gingivalis is closely associated to the initiation and progression of periodontal diseases. It also has various virulence factors, such as fimbriae, exopolysaccharides, proteinases, and hemin-binding proteins.[8] Epidemiological studies reveal that betel quid chewing, a common habit in Southeast Asia is linked with an increased risk of oral cancer and oral submucous fibrosis.[9] Studies have also shown a increased prevalence of periodontal disease among betel quid chewers than with nonbetel quid chewers.[10] Poor plaque control measures among betel quid chewers also describe the higher prevalence of periodontal disease.[10] However, at the same plaque level, betel quid chewers had a higher periodontal index than non-betel quid chewers.[11] This outcome suggests a direct impact of betel quid chewing on periodontal health, regardless of plaque infection.[11] The composition of betel quid differs in different geographic locations. However, in general it consists of piper betel leaf, areca nut (Areca catechu), and slaked lime with or without tobacco. Epidemiological and experimental studies reveal that the areca nuts have high potency of genotoxicity, carcinogenicity, embryo-toxicity and immune-toxicity.[12] The data supports that the areca nut extracts inhibited the growth, matrix protein synthesis and attachment of cultured gingival fibroblasts, claiming the concept that betel quid chewing may influence the periodontal health.[13] In addition, areca nut extract hampered the microbial mechanisms of neutrophils, proposing that areca nut extract might stimulate bacterial colonization and periodontal infection.[14] In vitro studies suggested that areca nut extract may also suppress the growth of some oral microorganisms.[15] However, the impact of betel quid chewing on oral microbial flora remains to be elucidated. Therefore, the aim of this

study is to quantify and compare circum-gingival porphyromonas gingivalis in betel nut chewers and non chewers.

MATERIALS AND METHODS

All the patients will be selected from the out patients section of The Department of Periodontology, KVG Dental College and Hospital, Sullia. A Written Informed consent will be obtained from all the patients participating in the study. Inclusion criteria are as follows: Patients will be selected from the age group of 30 -50 years, with absence of any systemic diseases, Previously periodontally untreated patients, Freedom from any dental and/or systemic pain or discomfort; Exclusion criteria are as follows: Medically compromised patient, Pregnant or lactating women, Physically and mentally challenged subjects, patients with antibiotic or using chemical plaque control agent. Ten subjects were included to determine the quantification of circum - gingival porphyromonas gingivalis in betel nut chewers and non chewers by real time PCR. Then evaluation and comparison of the amount of circum -gingival Porphyromonas gingivalis by real time PCR will be done in betel nut chewers and non chewers.

RESULTS

The real time PCR shows that there is no significant difference in the number of cells per ml among the groups. The study result shows that there is no association between the groups in the bacterial cell count.

DISCUSSION

A betel quid (BQ) is a blend of areca nut, slaked lime and powdered tobacco (occasionally) wrapped in piper betel leaf (Javed et al, 2013b). Other commonly added ingredients include gutka, menthol, and artificial perfumeries like musk ketones and sandalwood. Gutka is available commercially in colorful, glittery sachets even without health warning; whereas Betel Quid is available as non -branded pouches lacking health warning s (Figure 1). Both Gutka and Betel Quid are first placed in the mouth between the upper and lower posterior teeth, then gently chewed and sucked at irregular intervals. The stuffing of both forms is then held against the buccal vestibule over a long duration and is gently sucked continuously. The stuffing may either be sp it out or swallowed over time.

Studies (Javed et al, 2008, 2013a,b) have reported that periodontal inflammatory conditions [plaque index (PI), bleeding on probing (BOP), probing depth (PD), clinical attachment loss

(AL), and marginal bone loss (MBL)] are poorer in habitual gutka chewers and Betel Quid chewers with tobacco compared to individuals using non-tobacco products (non-chewers or controls). One justification derived from this is that all major ingredients of gutka and Betel Quid (such as areca nut, slaked lime, and powdered tobacco) have independent risk factors of oral gingival inflammation. Slaked lime leads to alkaline conditions in the oral cavity thus favoring the production of reactive oxygen species (ROS), which play a role in amplifying oral mucosal inflammation and carcinogenesis (Nair et al, 1990). Arecoline, an areca nut extracts impairs the gingival keratinocytes growth and the periodontal fibroblast function (Jeng et al, 1999); whereas ST has been reported to cause hyperemia in gingival blood vessels (Mavropoulos et al, 2001). It is appropriate to mention that the average weight of a gutka sachet is 3.5 grams (g) and the moisture content is 7%; while the estimated net weight of a Betel Quid is 4 g (with approximately 1.14 g of tobacco) and the moisture content is 70% (Babu et al, 1996). This reveals that gutka users consume more dry weight of tobacco, areca nut and slaked lime in comparison to Betel Quid chewers. However, betel quid also contains certain qauntities of tobacco, arecanut and slaked lime which can cause oral inflammation. To our data from indexed literature, there is no study on comparing and evaluating the circumgingival porphyromonas gingivalis between betel nut and non betel chewers.[16] So the aim of this study is to compare and evaluate the circum-gingival porphyromonas gingivalis in betel nut chewers and non betel nut chewers. Precise quantification on the number of individual bacteria present in plaque samples is required to understand properly the bacterial etiology of periodontitis.[8] Gram negative anaerobic bacteria present in subgingival plaque are the major factor which causes periodontitis. Around 400 bacterial species inhabitate human subgingival plaque.[17] Griffen AL et al. and Grossi SG et al.[18] in their studies have reported that Porphyromonas gingivalis as one of the main causative agents strongly involved in periodontitis. Though several species have been found in diseases individuals, a single species cannot be isolated from all periodontal patients, also apparently healthy subjects have been seen to harbor disease associated species.[19] Interaction between different species could be a reason for the formation of the disease.[20] The irreversible damage of the host tissue also results when the amount of bacteria reaches a critical threshold. Therefore, precise quantification of the amount of individual species of microorganism present is required to find the bacterial etiology of periodontitis. Commonly used sampling methods find out the different species of bacteria present but not the amount of bacteria present. Methods like immunoassay, cultivation and enumeration, and DNA hybridization have been used to quantify the periodontal pathogens present at sites of disease.[19] The studies done using

these techniques have provided useful data but could only find a small amount of organisms as potential pathogens. Whereas Real -time PCR with species specific primers can reproduce an accurate and sensitive technique for better quantification of entire bacteria as well as individual species.[14] Thus, Real -time PCR is a reliable method which provides an effective, sensitive and definitive approach to quantify these. The TaqMan system, which uses hydrolysis probes that are designed to rise the specificity of quantitative PCR are used to regulate the amount of Porphyromonas gingivalis and the total number of bacterial cells present in plaque samples.[8] The study results showed that no significant difference in the number of cells per ml among the groups. Table 1 shows that on evaluting and comparing porphyromonas gingivalis count between betel nut chewers and non chewers that there is no significant differences in both the groups. figure 1 shows the Bar chart showing the bacterial cell count per ml among the two groups in which there is no significant differences in it.

Table 1: Chi-square crosstabs for distribution of CFU cells in both groups.

No of bacterial cells	Group		Total	Chi-square statistic	P value
	BC	NBC			
Not detected	2	0	2	3.26	0.196
10 to10^4	2	1	3		
> 10^4	1	3	4		
Total	5	4	9		

Test: Fisher's exact test (Variant of Chi-square test for small samples)

Inference: The test shows that there is no significant difference in the number of cells per ml among the groups. The study result shows that there is no association between the groups and the bacterial cell count.

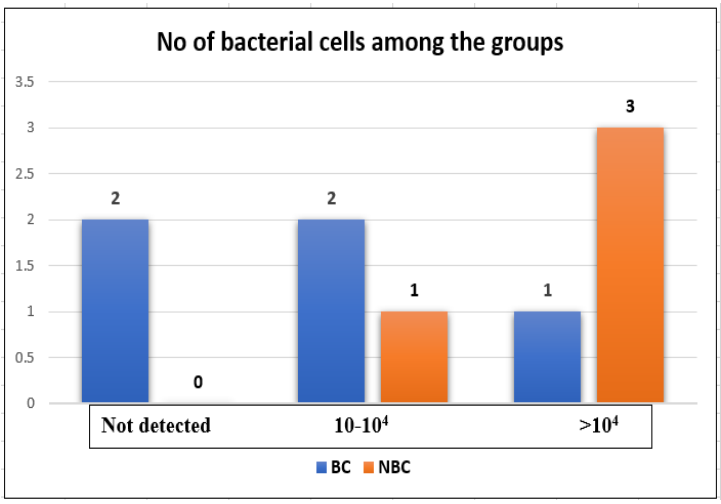

Figure 2: Bar chart showing the bacterial cell count per ml among the two groups.

CONCLUSION

The real time PCR test shows that there is no significant difference in the number of Porphyromonas gingivalis cells per ml among the betel nut chewers and non chewers groups in the circum gingival area. Hence it could be conclude d that betel nut chewing neither promote nor restrict the Porphyromonas gingivalis growth. Since this is the pioneer study evaluating and comparing porphyromonas gingivalis betweeen betel nut chewers and non chewers, further studies with larger sample size has to be carried out for even more accurate result.

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